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Response to Advisory Action of 4/22/04

**REMARKS**

Claims 1, 2, 8-13, 16 and 21-22 are rejected under 35 U.S.C. §102(e) as being anticipated by Portnoy et al. (U.S. Patent No. 6,004,815). Specifically, Portnoy is cited as teaching an attenuated derivative of a pathogenic microorganism, plasmid vectors, and a gene operably linked to a eukaryotic promoter. Further, Portnoy is cited as teaching E. coli deficient in the production of DAP and a recombinant complementing gene on a vector.

Applicant respectfully points out that the Office has failed to put forth a prima facie case of anticipation. The reference cited fails to teach each element of the rejected claims, as follows.

Claim 1 recites "an attenuated derivative of a pathogenic microorganism which comprises a) a non-functional native chromosomal essential gene; b) a recombinant complementing gene on an extrachromosomal vector, wherein the complementing gene can recombine to replace the non-functional native chromosomal essential gene; and c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding a desired gene product; wherein said complementing gene of b) is a functional replacement for said essential gene of a), wherein the desired gene is stably maintained in a progeny population of the microorganism." Claims 12, 45 and 46, the other pending independent claims, each recite an attenuated derivative of a pathogenic microorganism, and element (b), a recombinant complementing gene, wherein said complementing gene is a functional replacement for a non-functional

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native chromosomal essential gene. Thus, the arguments below are applicable to all of the rejected claims.

The Office has failed to identify any embodiment in the Portnoy reference that comprises a recombinant complementing gene on an extrachromosomal vector, where that complementing gene is a functional replacement for the essential gene of (a). The Office makes reference to various plasmids recited in Table 2 of Portnoy. Applicant maintains that none of the plasmids disclosed in that table, nor anywhere else in the specification comprises such a recombinant complementing gene. Plasmid pWR100, as discussed at column 16 does not provide a complementing gene, because the DAP deficient E. coli carrying the pWR100 plasmid spontaneously lyse in conditions where DAP is not available, thus indicating that the plasmid does not encode a gene that is a functional replacement for the defective gene which rendered the E. coli DAP deficient. If pWR100 carried a complementing gene, DAP would be produced such that the DAP deficient E. coli would not lyse. The recombinant complementing gene of claim 1 is a functional replacement for the essential gene, which essential gene is non-functional. In the case of a DAP deficient strain, the complementing gene must render the strain DAP+. pWR100 fails to accomplish this, as evidenced by the fact that the strains lyse, even when they carry the pWR100 plasmid.

Similarly, none of the other plasmids carry complementing genes, as recited in claim 1. pDP3615 carries a gene that encodes listeriolysin O. pDP3616 carries a gene that encodes chicken ovalbumin. pDH70 carries a promoterless lacZ gene. Each of these plasmids was inoculated into E. coli. None of those genes can possibly be

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considered complementing genes, because two of them are not *E. coli* genes, let alone essential *E. coli* genes. With respect to the strain into which pDP3615 and pDP3616 were inoculated, each plasmid carries a heterologous gene. LacZ is a well know reporter gene, and thus in the context of the rejected claims, is not an essential gene. None of the recited plasmids carry a gene that complements a non-functional native essential gene. The Office points to DAP as the essential gene, noting that the strains require DAP (for example, in culture medium) for viability. Applicant does not dispute this statement. However, nowhere does the Office point out any plasmid that carries a gene that encodes DAP. Again, element (b) of the instant claims recites "a recombinant complementing gene...wherein said complementing gene...is a functional replacement for said essential gene of (a)..." Thus, in order for any of the Portnoy embodiments to meet this limitation, the plasmids would necessarily have to comprise a gene encoding DAP. In other words, if DAP is the product of the "non-functional native chromosomal gene," then a plasmid would have to comprise a gene encoding DAP, thus "complementing" the DAP(-) phenotype of the strains, allowing them to survive in DAP-free media. None of the plasmids shown in table 2 or anywhere else in the specification, comprise such a complementing gene. The Declaration of Dr. Daniel A. Portnoy under 37 C.F.R. §1.132 provides further evidence of this insufficiency of Portnoy et al. as an anticipatory reference. As stated therein, none of the plasmids described in the '815 patent comprise a recombinant complementing gene, i.e., a gene that is a functional replacement for a non-functional native chromosomal essential gene.

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Further, the claims of the instant application are directed to "...[a] derivative of a pathogenic microorganism...." None of the microorganisms described in the Portnoy et al. reference meet this limitation. Portnoy et al. describes the use of non-pathogenic bacteria useful for delivery of agents to eukaryotic cells. See for example column 4, lines 26-29: "A wide variety of nonvirulent, non-pathogenic bacteria may be used; preferred microbes are relatively well characterized strains, particularly laboratory strains of *E. coli*, such as...." In addition, all of the bacterial strains utilized in the examples are derived from *E. coli* K-12, a well characterized non-pathogenic laboratory strain. Thus, Portnoy et al. fail to teach each and every limitation of the instant claims.

It appears that the Office has attempted to shift the burden of proving that Portnoy does not disclose the claimed compositions to Applicant. The Office cites In re Best and In re Fitzgerald et al. in support of that shift. Applicant respectfully points out that such a shift of the burden is improper in the instant case. Portnoy describes the plasmids and bacterial strains in sufficient detail that there is no legitimate question as to whether that reference teaches the claimed composition. In both of the cases cited by the Office, the Court is addressing functional claim limitations, and whether a prior art process inherently possesses that functional limitation. As the Court notes in In re Best "Where the Patent Office has reason to believe that a functional limitation asserted to be critical for establishing novelty in the claimed subject matter may, in fact, be an inherent characteristic of the prior art, it possesses the authority to require the applicant to prove that the subject matter shown to be in the prior art does not possess the characteristic relied on." In the instant case, the claim limitations in question are not functional

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limitations. Claim 1 recites a recombinant complementing gene on an extrachromosomal vector. It is apparent, upon a cursory reading of Portnoy, that the vectors described therein simply do not possess such a gene. The Office points specifically to pDP3615 as comprising "an RNA polymerase essential gene." The Office also points to the following text from the Portnoy patent, asserting that each element of the claims is taught: "In particular, the polynucleotide may encode a transcription factor, whereby expression of the transcription factor in the target cell provides activation or de-activation of targeted gene expression in the target cell." That phrase refers to a transcription factor as an example of a nucleic acid based agent that can be delivered via the microbial delivery vehicles disclosed in that reference. Applicant does not dispute that Portnoy teaches delivery of nucleic acid based agents, including a variety of genes, as discussed at column 3, lines 39-60. The instant claims, however, recite a complementing gene, wherein said complementing gene is a functional replacement for the non-functional native chromosomal essential gene. Nowhere does Portnoy teach or even suggest providing a functional replacement for a non-functional native chromosomal gene, on an extrachromosomal vector. Claim 1 also recites "...[a] derivative of a pathogenic microorganism...." As discussed above, Portnoy teaches derivatives of non-pathogenic microorganisms. Again, only a cursory reading of Portnoy et al. is required to make such a determination. The Office has thus failed to identify each and every element of the claims in the cited reference. Therefore, Applicant respectfully requests reconsideration and withdrawal of the rejection.

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Claims 1-7, 12-20 and 45-46 are rejected under 35 U.S.C. §102(e) as being anticipated by Curtiss III et al., U.S. Patent No. 6,024,961. The Office makes reference to Applicant's previous response, in which much of the argument focused on U.S. Patent No. 5,672,345, and suggests that such arguments are not relevant. Applicant respectfully points out that the '345 patent is discussed because at column 42, line 1, of the '961 patent, the '345 patent is incorporated by reference as teaching the construction of an *asd*-complementing plasmid. That *Asd*<sup>+</sup> plasmid is utilized to supply a complementary *asd* gene to a bacterial strain that is *Asd*<sup>-</sup>. The argument in Applicant's prior response focuses on the fact that the complementary gene taught in the '345 patent, incorporated by reference into the '691 patent, is incapable of recombining to replace the non-functional native chromosomal essential gene. Applicant respectfully points out that that argument is directly on point, and it is reiterated here. All of the examples in the '961 patent utilize a complementing gene that cannot recombine to replace the non-functional native chromosomal gene. At column 42, lines 59-61, it is suggested that a vector may be constructed such that the vector will insert into the bacterial chromosome by homologous recombination or by transposition. That comment refers to a single reciprocal crossover event, by which the circular plasmid DNA is integrated into the larger circular chromosomal DNA. In such a case, the wild-type essential gene (integrated into the chromosome via the single crossover event, as above) and the non-functional chromosomal gene will both still be present. Such recombination, if it occurred, would not permit replacement of the non-functional chromosomal essential gene as recited in the instant claims. Replacement requires two

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reciprocal crossover events, and both of those events demand as an obligate condition, the existence of homologous sequences, on the vector and in the chromosome, that flank the recombinant complementing gene and the non-functional native chromosomal essential gene. Since the '961 patent does not disclose a recombinant complementing gene on an extrachromosomal vector, *wherein the complementing gene can recombine to replace the non-functional native chromosomal essential gene*, that patent cannot anticipate the instant claims. Therefore, applicant respectfully requests reconsideration and withdrawal of the rejection.

All of the embodiments of the balanced-lethal host-vector system constructed prior to the experiments described in the instant application have been constructed in such a way that the recombinant complementing gene *cannot* recombine to replace the non-functional native chromosomal gene. For example, the '345 patent (incorporated by reference into the '691 patent) describes the generation of  $\Delta$ asd mutation by excision of a Tn10 linked closely to the asd gene. The mutation is due to Tn10 insertion into a DNA sequence adjacent to the asd gene, and then the  $\Delta$ asdA1 mutation arose by fusaric acid resistance selected deletion of the Tn10 and adjacent gene sequences that included the asd gene. As a result, DNA sequences flanking the asd gene were deleted also. Thus, it would be impossible for a wild-type asd gene, such as is carried on plasmid pYA3148 or pYA3193 to recombine to replace the deleted chromosomal asd gene. The prevailing theory at that time (a theory that the Office adopted during prosecution of the '961 patent), was that if the flanking sequences (on either side of the asd gene) were left intact, the recombinant asd gene could recombine to replace the

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deleted chromosomal gene, thus rendering the balanced-lethal host-vector system inoperative. The Examples in the instant application provide the first evidence that contradict that premise. Here, Applicant has shown, by utilizing techniques of generating defined deletion mutations by allele replacement, the structural *asd* gene may be deleted without affecting the flanking sequences. Thus, when *asd*<sup>+</sup> vectors that include the *asd* gene and its flanking 5' promoter and 3' termination sequences are used to complement the *asd* chromosomal deletion, it is in theory possible for that gene to recombine to replace the deleted chromosomal gene. See Figure 11 of the instant specification, where examples are provided illustrating the extent of sequences encoding the wild-type *asd* gene and its 5' and 3' flanking sequences as found on various *Asd*<sup>+</sup> vectors and the extent of deletions depict the possible regions in which recombination between homologous sequences on the vector and the chromosome might occur. Such recombination, if it occurred readily and frequently, would render the system inoperative.

Prior to the experiments described in the instant application, no examples were known where it is possible for the complementing gene to recombine, as discussed above. Thus, the limitation in the instant claims, i.e., "wherein the complementing gene can recombine to replace the non-functional native chromosomal essential gene" is not found in any of the cited prior art. Applicant has discovered, surprisingly, that such a system is in fact functional. The claims recite such a system.

The Office further asserts that Applicant is arguing limitations that are not in the claims. As discussed above, the claims recite "a recombinant complementing gene on

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an extrachromosomal vector, wherein the complementing gene can recombine to replace the non-functional native chromosomal essential gene..." As shown above, that limitation is not taught by the prior art of record. Thus, because each element of the claims is not taught by the cited art, Applicant respectfully requests reconsideration and withdrawal of this rejection.

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action, and as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, he is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,



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